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Application of Plant Tissue Culture to the Propagation of Forest Trees

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Intensive culture of short rotation forest crops or the large scale replanting of forest land with selected, superior trees will depend on techniques for their practical, rapid, clonal, mass propagation. Standard methods of vegetative propagation can be used to multiply planting stock of some species, but they are not successful with many woody plant species. Plant tissue culture could provide a means for the clonal multiplication of selected individual trees in large numbers, in a short period of time and in a relatively small space. Techniques developed for tissue culture propagation of herbaceous, horticultural plants have now been successfully applied to one group of economically important woody plants, the poplars.

"Classical" plant tissue culture involves the initiation and growth of callus tissue in culture. Callus tissue does not correspond to any normally occurring plant tissue but it is most similar to the wound tissue that forms as a result of injury to the plant. Callus typically consists of parenchyma cells that grow in an unorganized mass. Callus tissue formed from tobacco stem pith cells has for many years been used to study the effects of certain chemical compounds on plant growth and development.

Auxins and cytokinins are two of the major types of plant growth regulating compounds found naturally in plants. Auxins stimulate the elongation of plant cells without an increase in the number of cells. Cytokinins stimulate the division of cells which results in an increase in the number of cells without an increase in their size. By controlling the ratio of auxin to cytokinin the growth of the callus tissue can be regulated.

When auxins alone are incorporated into standard plant tissue culture media at certain concentrations, they stimulate the formation of roots on callus of tobacco pith. Treatment with cytokinin at certain concentrations will stimulate the formation of shoots on tobacco pith cells. Sequential treatment first with cytokinin to initiate shoots and then with auxin to initiate roots can produce a complete plant from callus. Callus tissue derived from many species of plants, including several hardwood and a few coniferous tree speices behave similarly.

These experiments demonstrate totipotency, or the theory that every cell within a plant has the ability, under the proper conditions, to produce a complete plant. Certain cells within a plant, however, may be able to express their totipotency more readily than other cells. The expression of totipotency seems to depend on the particular cell type and its location within the plant. Variation in the ability to express totipotency seems to be caused by blocking of a specific portion of the cell's genetic information necessary for the expression of totipotency.

The production of shoots and whole plants from callus has not been widely applied to the propagation of plants for two main reasons. First, shoots are usually produced either singly or in low numbers from each piece of callus. Thus, the production of the large number of plants necessary to make tissue culture propagation practical is usually not possible using callus cultures. A second major problem with the use of callus cultures for propagation is that callus may not remain genetically stable when grown for extended periods of time in culture. In normal cell division the chromosomes double and divide with the rest of the cell to produce two daughter cells. Cells grown in culture commonly undergo a duplication of the chromosomes without the division of the cells. As a result, the chromosome number of the cells doubles. Thus, diploid (2n) cells of a freshly initiated callus may become tetraploid (4n), hexaploid (6n) and eventually polyploid (many n) the longer the cells are kept in culture. Polyploidy may result in the loss of the cells' ability to express their totipotency and as a result the callus may not be able to form shoots or roots. Perhaps more importantly, polyploidy may result in the formation of plants that are genetically different from the parent tree and thus its desirable traits may be lost.

To determine if these techniques could be applied to woody plants, I collected shoot tips of the poplar clone Tristis #1 from an experimental plot north of Ames in mid-August. The shoots were brought back to the laboratory where they were dissected down to a shoot apex 1-2 mm tall. Each apex was then placed in a test tube containing a medium for their growth which is basically a nutrient solution consisting of inorganic salts, several vitamins, different combinations of auxin and cytokinin, sugar as a energy source and a gelling agent solidify the medium. The different

combinations of different concentrations of auxin and cytokinin were tested to determine the concentrations best for the multiplication of shoots. One apex was planted per tube and they were then placed in a growth chamber under controlled light intensity, photoperiod and temperature.

After 4 to 6 weeks in culture, several of the apices had developed small buds on the callus that formed at the base of the original shoot apex (Figure 1). The various auxin-dytokinin combinations differed greatly in their ability to produce these adventitious buds. One particular combination resulted in the formation of between 8 and 12 of these adventitious buds from each original apex (Figure 2). This particular combination was selected as the shoot multiplication medium to be used in all further experiments with Tristis #1. As these buds grew, they developed into a mass of shoots that surrounded the original apex (Figure 3). At this time I could separate this mass of shoots into individual shoots and either place them on a fresh tube of multiplication medium or on a rooting medium.

If I placed the shoots on a fresh tube of multiplication medium, they could produce another 8 to 12 adventitious shoots at several points along their edge within 4 to 6 weeks (Figure 4). The rooting medium consisted of the same basic medium as the multiplication medium except that it contained high levels of auxin to stimulate root formation and contained no cytokinin. If individual shoots were placed on the rooting medium, a normal looking root system complete with lateral roots formed within 2 to 3 weeks (Figure 5). After the roots began to appear, I removed the plants from their containers, washed the medium from the roots and planted them in peat pellets under a shaded mist system in the greenhouse to condition them slowly to the environment outside the test tube.

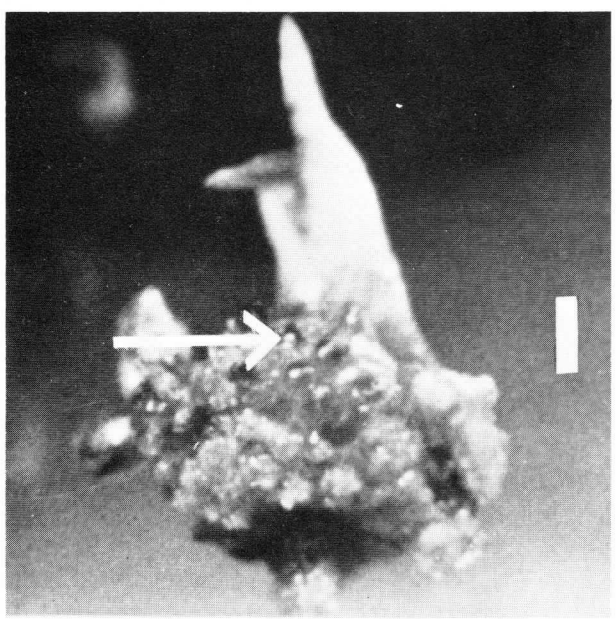

Figure 1. Poplar Tristis #1 shoot apex after 4-6 weeks in culture. Arrow indicates the meristematic areas developing on the callus at the base of the original shoot apex. Scale equals 1 mm.

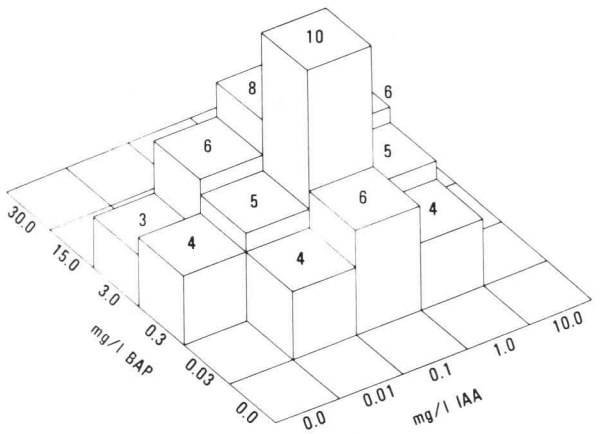

Figure 2. Average number of adventitious shoots produced on excised poplar apices placed on the 30 different auxin (IAA) and cytokinin (BAP) combinations (mean of three replicates).

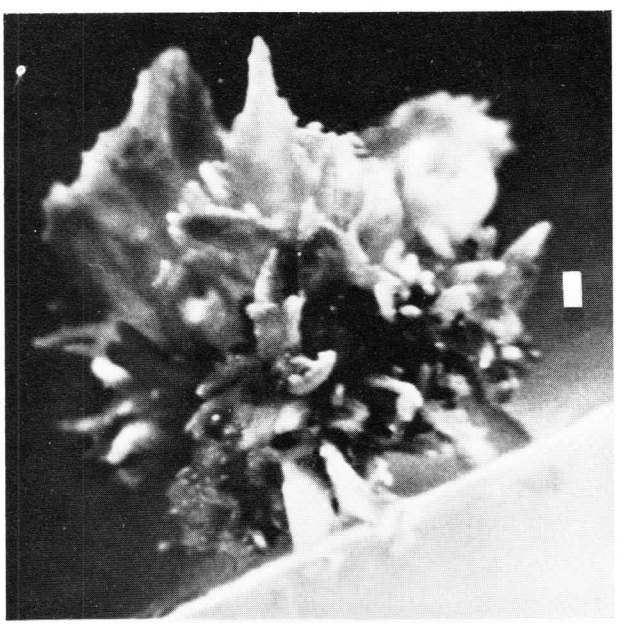

Figure 3. Mass of leaves produced from the adventitious buds in figure 1 after 6-8 weeks in culture. Scale equals 1 mm.

Although most poplars are easily propagated through traditional techniques of vegetative propagation, shoot apex propagation offers several advantages to the plant propagator. First, using standard shoot tip cuttings, each Tristis #1 shoot tip will produce only one plant. Using shoot apex

culture, each Tristis #1 shoot apex can produce between 8 and 12 shoots each of which can be rooted to produce a complete plant or if the individual shoots are placed on fresh multiplication media, each one will produce another 8 to 12 shoots. Thus, with each such transfer to fresh multiplication media, the number of plants produced increases geometrically. It is in this way that one million Gerbera daisy plants can be produced from one original shoot apex in only one year.

A second advantage of shoot apex propagation is that it does not require a large amount of space. The growth and multiplication of shoots takes place in test tubes grown on shelves illuminated from above by fluorescent lamps mounted on the bottom of the shelf above. In this way it has been estimated that a room 13' x 13' x 9' could contain about 10,000 tubes and in the case of Tristis #1 each tube would contain between 8 and 12 shoots, each capable of producing a complete plant. An equal amount of space in the greenhouse could support 150-200 stock plants, each capable of producing 10 to 20 shoot tip cuttings. Obviously shoot apex propagation is capable of producing many more propagules in a given space. Greenhouse space in an apex propagation system would be necessary only for the maintenance of stock plants and for a mist system where the rooted plants are grown before planting out.

Third, shoot apex propagation could be used in the production of pathogen-free plants. For standard multiplication purposes the apex is dissected down to 1-2 mm tall and consists of the apical dome and several leaf primordia. For a pathogen-free plant the apex is further dissected down to between 0.05-1.0 mm tall and consists only of the apical dome. The use of the smaller apical dome makes possible the production of plants free of fungi, bacteria, viruses, viroids, mycoplasmas, spiroplasmas and rickettsias. These organisms cause plant diesases and restrict the growth and productivity of the host plant. The apical dome is one of the few parts of the plant that may be free of these organisms. Pathogen-free plants may exhibit an increased growth rate for a time after they are planted in the field, until they become naturally reinfested. The increased growth rate after planting, however, may be critical to their survival and subsequent growth. Plants so produced could also meet the pathogen-free requirements encountered in the international exchange of plant material, an important component of many tree improvement programs.

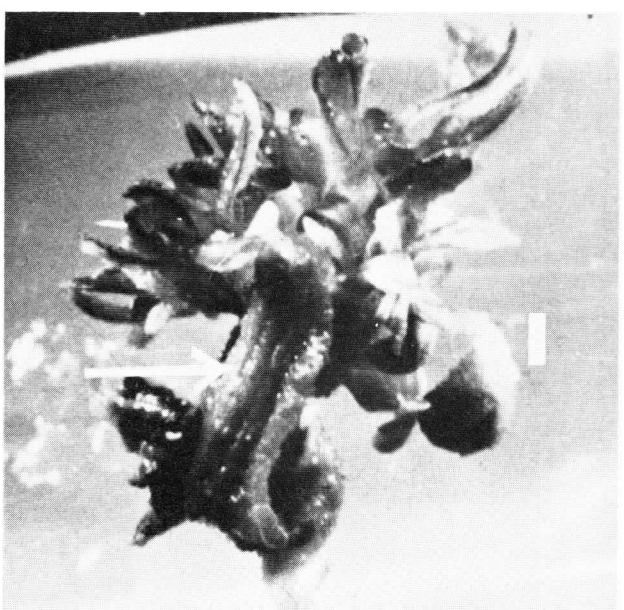

Figure 4. An adventitious shoot separated from a mass of shoots and placed on fresh multiplication media after 2-3 weeks. Arrow indicates the original adventitious shoot with new adventitious shoots developing from its edge. Scale equals 1 mm.

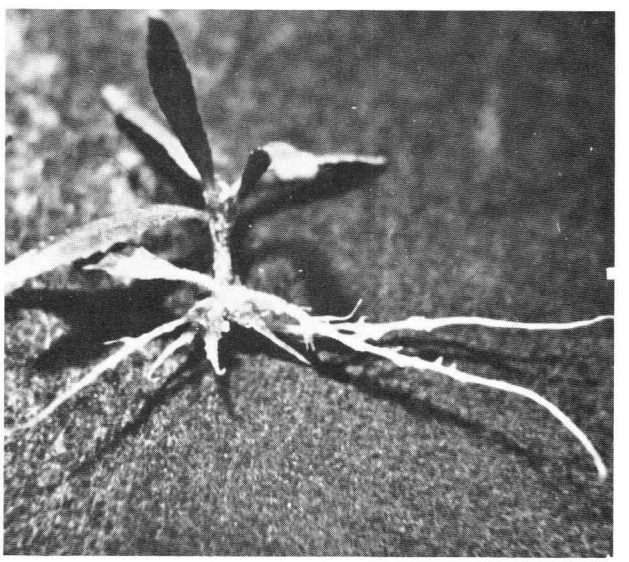

Figure 5. An adventitious shoot after 3 weeks on a rooting medium showing the formation of a root system with lateral roots. Scale equals 1 mm.

Tissue and shoot apex culture at this time appear to be easiest with species of herbaceous and woody plants whose cutting can be rooted easily. The experimental work reported here with poplar demonstrates that the basic techniques developed for herbaceous plant propagation via shoot apex culture can be successfully applied to at least some woody genera. Perhaps with further experimentation these techniques can be applied to other woody genera. The development of a similar propagation system for the conifers would be of

great importance because most are difficult to propagate vegetatively.

Vegetative propagation makes possible the multiplication of plants before they reach the age of flowering and seed production and in trees this can mean a substantial saving in time. It also ensures that the trees produced will be identical genetic copies containing the desired traits of the original selected tree. With seed propagation, the resulting trees usually will not be identical to the parent tree. Shoot apex propagation has a potential for the large scale, clonal propagation of selected trees. It has the advantage over standard methods of vegetative propagation in the production of a large number of identical plants in a short period of time and in a relatively small amount of space. The availability of mass propagation systems for select, superior trees would help make possible their use in large scale intensive silvicultural systems as well as large scale replanting of forest land with superior trees.

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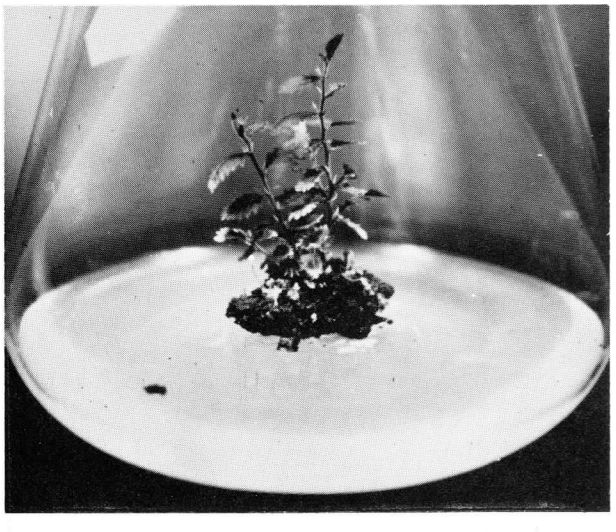
A native of Staten Island, New York, Mr. Thompson received a Bachelor's degree in biology from Wagner College in 1971. He received a Master's degree in botany and plant pathology from Iowa State in 1974. Since that time he has been employed as a Research Associate with the Department of Forestry. His main research interest is in the tissue culture of plants, in particular woody plants, and its application to tree propagation and studies of the physiology of tree growth and development.



Test Tube "Seed" Orchards!?

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Introduction

The preceeding paper by David Thompson has explored the potential for mass producing selected trees by tissue culture. Here I will suggest using tissue culture in the processes of tree breeding and "seed" production. A few years ago the first plant hybrid was produced by a tissue culture technique called parasexual hybridization. Leaf cells were isolated from two species of tobacco, their cell walls were enzymatically removed, and they were induced to fuse into new hybrid cells that could be carried on through tissue culture techniques to become new hybrid plants (3). In this paper I want to suggest that an adaptation of this method for bypassing sexual reproduction could become the method of choice in the breeding of some pine species. My argument to this effect will consist of two parts: 1) Problems with the current approach to tree improvement that might be solved by the new approach, and 2) special features of the pines that might fit them especially well to the new technique. Some of the techniques and advantages described could also be applied to other conifer genera.

Current Breeding Procedures for Pines

Different pine species reach reproductive maturity at anywhere from 2 years under intensive culture to more than 50 years in some high latitude regions. On a normally growing pine the reproductive structures, especially the female cones, are produced well out of efficient research for the forest geneticist. Selected parental material for a pine breeding programs may be growing at widely separated locations; indeed, some interspecific breeding programs require parental material from different continents. Consequently, the first step in a pine breeding program is the establishment of a breeding or seed orchard where all the selected